

**Marked-up version of specification**

instructed in the manufacturer protocol (Qiagen Catalog No. 12143). The positive clones then are sequenced using a T7 forward primer (5'-TAATACGACTCACTATAGGG-3') (SEQ ID NO:8) and an M13 reverse primer (5'-CAG[U]TAAACAGCTATGACCAT-3') (SEQ ID NO:9). DNA sequencing 5 identified isolation of a cDNA having the DNA sequence presented in Figure 1 (SEQ ID NO:1) and the amino acid sequence presented in Figure 2 (SEQ ID NO:2).

**Example 2-Generation of Mammalian Cells Overexpressing hGAVE3**

10 To provide significant quantities of hGAVE3 for further experiments, the cDNA encoding hGAVE3 is cloned into an expression vector and transfected into mammalian cells, such as 293 cells.

15 To generate mammalian cells overexpressing hGAVE3, mammalian cells are plated in a six-well 35 mm tissue culture plate ( $3 \times 10^5$  mammalian cells per well (ATCC Catalog No. CRL-1573)) in 2 ml of DMEM media (Gibco/BRL, Catalog No. 11765-054) in the presence of 10% fetal bovine serum (Gibco/BRL Catalog No. 1600-044).

20 The cells then are incubated at 37°C in a CO<sub>2</sub> incubator until the cells are 50-80% confluent. The cloned cDNA nucleic acid sequence of hGAVE3 is inserted using the procedure described above in a pcDNA 3.1 cloning vector (Invitrogen, Catalog No. V790-20). Two  $\mu$ g of the DNA are diluted into 100  $\mu$ l of serum-free F12 HAM media. Separately, 25  $\mu$ l of Lipofectamine Reagent (Life Technologies, Catalog No. 18324-020) is diluted into 100  $\mu$ l of serum-free F12 HAM media. The DNA 25 solution and the Lipofectamine solution then are mixed gently and incubated at room temperature for 45 minutes to allow for the formation of DNA-lipid complexes.

30 The cells are rinsed once with 2 ml of serum-free F12 HAM media. For each transfection (six transfections in a six-well plate), 0.8 ml of serum-free F12 HAM media are added to the solution containing the DNA-lipid complexes (0.2 ml total volume) and mixed gently. The resulting mixture (hereinafter the "transfection mixture") then is overlaid (0.8 ml + 0.2 ml) onto the rinsed cells. No anti-bacterial